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A HERITABLE BLOTCH LEAF IN OATS

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A HERITABLE BLOTCH LEAF IN OATS

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THE present work deals with a spot necrosis, not formerly described, in a Norwegian variety of oats which for a series of years has been cultivated at experiment stations in Sweden and Denmark. The disease, which we name »Blotch Leaf», at a first glance conveys an impression of being caused by an unfavourable condition of the soil or by a parasitic attack, but it is, in fact, of a genetic nature, as it is capable of being transmitted from one generation to the other both through eggs and through pollen.

Our material originates from the Plant Pathological Experiment Station at Lyngby, Denmark, where in the years 1910—1925 a variety of oats with a so-called »heritable dry speck disease» was cultivated. An examination of the experiment records showed that the variety had been received from the Swedish Experiment Station at Svalöf in 1910 under the designation of »hvitkornig yfvippa 0540» (i. e. a white-grained oat with a spreading meagre panicle with long branches and slender grains).

From the descriptions in the experiment records of Svalöf from the years 1898—1903 it appears that the variety described here really corresponds to that of ours. From the characteristics of the variety the following may be emphasized: Long, slender spikelets; yellow husks; kernel greyish-yellow and light in weight; the palea provided with a long geniculate, twined awn; lower flower long-haired at the base; branches of the panicle rather long and lax.

According to statement of Dr. Å. ÅKERMAN of Svalöf this variety of oats is a pedigree of an oat from Nordfjord in Norway (1200 feet above the sea). In the experiment records of Svalöf for 1904 Professor NILSSON-EHLE has stated that the variety most likely belongs to the »Snaasenhavre» group.

Curiously enough the Swedish experiment records do not tell us that the variety is always blotched in the first part of its period of growth (cf. below); but this is presumably to be explained from the fact that the observations on the variety were not kept till the plants had eared.

That the blotching has been observed already at Svalöf appears, however, from T. HEDLUND's article (1913), presumably the only literary treatment in existence concerned with our blotch leaf. HEDLUND's statement at large, translated from Swedish, runs thus: »In the botanical experiment garden here (Alnarp, Sweden) a white-grained variety of oats with long, slender kernels is cultivated, which is known by the fact that the leaves of the young plants get rather large red-edged spots of dead tissue. This leaf spot disease, which somewhat reminds of the dry speck disease of oats, makes its appearance under any circumstances, even if the variety is cultivated in the greenhouse in sterilized soil. I have also from Svalöf been informed that the spots belong to the variety».

SYMPTOMS OF THE DISEASE.

The disease, which practically attacks all the individuals of the variety concerned, appears at an early stage and is chiefly observed on the lower leaves, more especially on the lowest 3 or 4 leaves. The higher placed leaves are generally free from the attack, which does not prevent the plants from earing and fructifying. The first symptoms of the disease turn up as greenish brown, diffuse spots, which gradually through the necrotisation of the tissue are coloured from pale-brown to nutbrown with a reddish brown tinge, or in some cases greyish pale. The faded parts are surrounded by a more or less distinct, more intensely coloured zone, which especially on the lower side of the leaves can be from reddish brown like mahogany to purple-brown. Sometimes there are on each leaf only some few elongated spots, from $^1/_2$ to 2 cm. long, sometimes numerous ones, which then often are arranged in stripes and merged together, because of which larger or smaller parts of the blade fades. The lower, first attacked leaves are most severely damaged and frequently show faded border or cross zones. The general character appears from Plates II and III.

By the brownish colour, which the spots assume in typical cases, and by their arrangement not strictly stripe-wise, the disease is generally easily known from the dry speck disease of oats (German: Dürrfleckenkrankheit; Dutch: Veenkoloniale Haferziekte), which according to recent investigations is due to the influence of a poisonous humic substance (See Text-Figs. 1 and 2).

Where only small, separated, reddish brown spots with an intensely coloured border appear, the symptoms may remind of late phases

Fig. 1. Leaves of the blotched oat-variety.

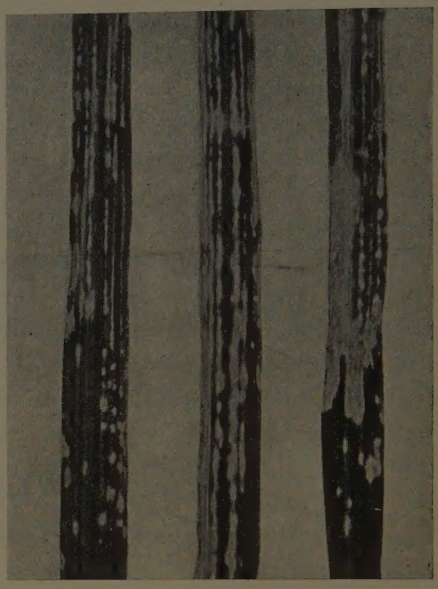

Fig. 2. Leaves of an oat-variety (»Segerhavre») showing dry speck disease.

in the attack of *Septoria avenae*; the intense, crimson colour, which always accompanies this disease, has, however, here, as above mentioned, been supplanted by brownish shades.

EXPERIMENTAL INVESTIGATIONS ON THE GENETIC NATURE OF THE BLOTCH LEAF.

The mere circumstance that the disease always accompanies the given variety of oats, and that it has not been traceable in numerous others which we have had an opportunity to observe, must, of course, convey an anticipative supposition that the disease is of a genetic nature; this is further confirmed by the fact that it has proved impossible for us to point out pathogens in connection with blotch leaf, as also by the fact that the disease evidently appears independently of the condition of the soil.

But the possibility had still to be considered that it might be a case of a so-called »virus disease». This supposition is, however, opposed by the following facts: (1) Virus diseases in plants are always characterised by a great infection power, but, as above mentioned, this disease never spreads to other varieties of oats even if these are cultivated side by side with our variety year after year. — (2) Virus

diseases always produce a spotting with yellow spots or bleaching of the attacked parts of the plant, without their being necrotized. — (3) Although it can hardly be regarded as finally ascertained whether virus diseases can be transmitted by seed, yet the existing statements are to the effect that this is only exceptionally the case, — and here we have just a typical transmission both through eggs and through pollen. — (4) An experiment from 1925 of transmitting blotch leaf from sick plants to a sound »Segerhavre» by means of plant-lice, which were gathered on sick leaves and confined on »Segerhavre» in thin parchment bags turned out a failure in spite of the fact that the lice throve and multiplied very well during the confinement.

All told it thus seemed that only one possibility was left, namely that our blotch leaf must be of a genetic nature, and our endeavours so must be to prove this through experiments of crossing. The experiments were undertaken at the Genetic Experiment Station of the Royal Veterinary and Agricultural College at Lyngby near Copenhagen.

The P-Generation. — In 1922 we sowed in the greenhouse partly the blotched variety, partly a sound oat, namely »Segerhavre», which is a variety produced by the Swedish Experiment Station at Svalöf, and in 1923 the grains of the material thus controlled were sowed in the open. As might be expected the two varieties were completely constant, all individuals of the sound variety being non-blotched, while all individuals of the sick variety were blotched (See Plate II, Fig. 1 and Text-Fig. 3, leaves Nos. *1*—*4*), and thus the relation remained through the whole period of the experiments, in which the two *P*-types were constantly cultivated in lots situated immediately beside the crossing-material.

Of the characteristics of the two varieties the following may be emphasized: Sound variety (»Segerhavre»): Stub spikelets, no hairs at the base of the flowers, a very short awn at the lower flower. Sick variety: Rather slender spikelets, long hairs at the base of the lower flower, the palea of which is provided with a long, geniculate, twined awn.

In July 1923 an individual of each variety was planted in pot in the greenhouse and some reciprocal crossings of the two individuals were undertaken, 6 flowers of the non-blotched plant after emasculation being pollinated with pollen from the blotched one, and 7 flowers of the blotched plant in the same way being pollinated with pollen from the non-blotched one. From the crossing: non-blotched ♀

× blotched ♂ only 1 kernel was produced, while the crossing: blotched ♀ × non-blotched ♂ produced a total of 5 kernels.

The F_1-Generation. — The 6 kernels (total of the kernels produced in the crossing) in 1924 produced 6 F_1 plants, which were cultivated in pots in the greenhouse. The morphological examination of these

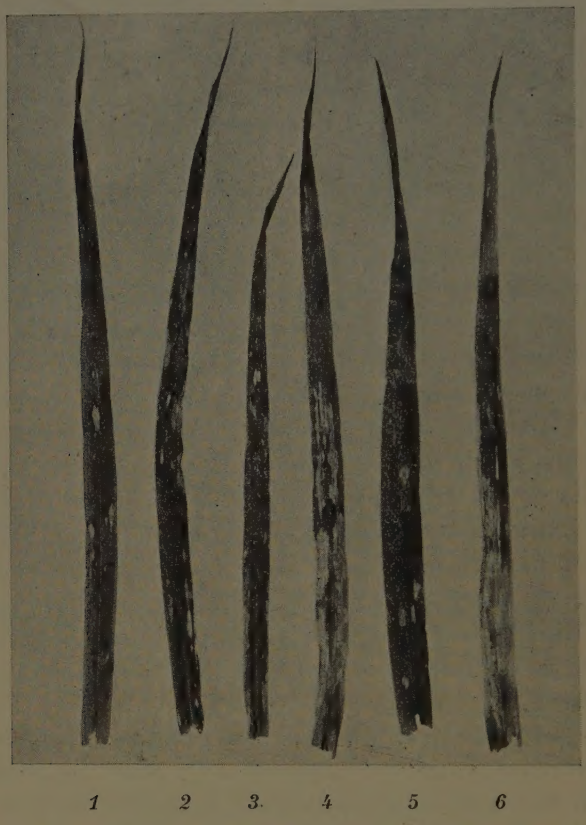

Fig. 3. Leaves nos. *1—4* from the blotched oat-variety; leaf no. *5* from an F_2-plant, the disease of which is transferred to F_1 through the egg (Pedigree no. 2453); leaf no. *6* from an F_2-plant, the disease of which is transferred to F_1 through the pollen (Pedigree no. 2447).

plants clearly showed their hybrid character. There were no long hairs at the base of the lower flowers, but at the most 2 or 3 short hairs on some few of these, while the awn was well-developed although hardly so long and so greatly twisted as in the blotched variety. Morphological differences between the reciprocally produced hybrids could not be pointed out.

As for the character of the blotch it can be stated that the only produced F_1 individual of the crossing non-blotched ♀ × blotched ♂ was not visibly blotched while 2 individuals of the reciprocally produced F_1 plants proved somewhat blotched. (See Plate III, Fig. 3). It should be noted that the circumstance that the F_1 plants were cultivated in the greenhouse may probably have had some influence on the rather scarce appearance of the disease.

All the 6 F_1 individuals throve very well and after being isolated were left to self-pollination; when ripe the kernels were gathered separatedly from each plant.

The F_2-Generation. — The 6 F_2 progenies were sowed in 1925 and proved all of them to include both non-blotched and blotched plants, although in somewhat varying numerical relations.

The only F_2 progeny after the crossing non-blotched ♀ × blotched ♂ showed the following numerical relations:

		Offspring		
Pedigree No.	Phenotype of mother plant: non-blotched	non-blotched	blotched	total
2447		341	67	408

A blotched plant of this pedigree is pictured in Plate II, Fig. 2.

The 5 F_2-progenies after the crossing blotched ♀ × non-blotched ♂ showed the numerical relations shown below:

		Offspring		
Pedigree No.	Phenotype of mother plant	non-blotched	blotched	total
2449	non-blotched	244	83	327
2451	blotched	296	116	412
2453	non-blotched	354	130	484
2457	blotched	245	105	350
2459	non-blotched	246	117	363
	Total	1385	551	1936

A blotched plant of pedigree No. 2453 is pictured in Plate III, Fig. 4.

With regard to the numbers above it is to be emphasized that only such plants as with full security were blotched are given in the category of blotched. Among these there were again a good deal of variation in the grades of the symptoms. Enumerations were under-

taken several times in the period of growth, as we very soon learned that the disease manifests itself with an unequal rapidity in each separate individual; for the successive countings showed an increasing number of blotched plants — although only up to a certain moment, as the disease rarely appears higher than on the fourth leaf. To give a concrete instance:

Pedigree No. 2451 sowed $^{22}/_{4}$ 1925. Allt plants had eared $^{30}/_{6}$.
Enumeration $^{24}/_{5}$: 368 non-blotched, 13?, 31 blotched
» $^{13}/_{6}$: 296 » 0?, 116 »

The plants recognized as blotched were in the enumeration marked with a red thread. Although attacks from *Septoria* and plant-lice, or other influences, which discolour the leaves of oats, of course, to some degree might render a sure diagnostication of faint blotch symptoms difficult, we dare all the same maintain that the enumeration in the main offers a true picture of the relations, as the doubtful cases were very few.

From the enumerations in the F_2 material the following conclusions may be drawn: Both eggs and pollen are able to transmit the disease to the progeny, although this obviously takes place to an unequal degree, and so that the disease is more easily transmitted through eggs than through pollen. Where the disease is transmitted to F_1 through pollen (Pedigree No. 2447) the result in F_2 is 341 non-blotched and 67 blotched plants, while at the transmission of the disease to F_1 through the egg, the result in F_2 is 1385 non-blotched and 551 blotched plants. If these results are added together, we get 1726 non-blotched and 618 blotched plants, which might lead one to think of a 3 : 1 segregation. That this is out of the question is proved, partly by the greatly varying relations of each separate pedigree, partly by an analysis of the F_3 and the F_4 generations.

The F_3-Generation. — As the starting-point for the production of the F_3 generation we chose partly 6 F_2 individuals of the Pedigree No. 2447 (disease transmitted to F_1 through pollen) and partly 6 F_2 individuals of the Pedigree No. 2451 (disease transmitted to F_1 through eggs).

The following tables give a survey of the result of the F_3 enumerations (see page 171).

A glance at the numbers of the enumerations shows that this is not a case of Mendelian segregations; sometimes a large surplus of blotched individuals is segregated, sometimes one of non-blotched individuals, and apparently there is no pronounced correlation between

F_3 enumeration (disease transmitted to F_1 through pollen). Date of sowing: $^{22}/_4$ 1926. Date of enumeration: $^{17}/_6$.

			Offspring		
Pedigree No.	Mother plant	Phenotype of mother plant	non-blotched	blotched	total
3180	2447—1	much blotched	34	71	105
3181	2447—2	very much blotched	8	34	42
3182	2447—3	»	63	112	175
3183	2447—4	much blotched	152	19	171
3184	2447—5	»	1	75	76
3185	2447—6	somewhat blotched	27	16	43
		Total	285	327	612

F_3 enumeration (disease transmitted to F_1 through eggs). Date of sowing: $^{22}/_4$ 1926. Dates of enumeration: $^{17}/_6$ and $^{21}/_6$.

			Offspring		
Pedigree No.	Mother plant	Phenotype of mother plant	non-blotched	blotched	total
3186	2451—1	much blotched	14	154	168
3187	2451—2	rather much blotched	8	103	111
3188	2451—3	somewhat blotched	2	28	30
3189	2451—4	much blotched	5	190	195
3190	2451—5	somewhat blotched	17	23	40
3191	2451—6	»	5	60	65
		Total	51	558	609

the grade of blotching of the mother plant and the character of the progenies, although the two most blotched F_3 families (Pedigrees No. 3184 and No. 3189) are both descended from much blotched mother plants.

It is remarkable, too, that, when a comparison of the summed up numbers is made, the highest degree of blotching as in F_2 appears in the material to which the disease is originally transmitted through the egg.

The F_4-Generation. — In order to try whether it is possible through a continued selection in the most blotched families to produce lines of mere blotched plants, individuals of the above pedigrees No. 3184 and No. 3189 were selected with the result that the whole pro-

geny became blotched, namely of the Pedigree No. 3184 a total of 164 plants and of the Pedigree No. 3189 222 plants.

DISCUSSION.

The experimental results numerically expressed in the above tables allow of drawing the definite conclusion that this is not a case of a Mendelian segregation, and so there is no disease-producing gene attached to the chromosomes. The element that transmits the disease from one generation to the other is consequently to be sought in the cytoplasm, i. e. in the plasm itself or in its plastids.

This again offers the following possibilities:

(1) The element of the disease might be a virus. This hypothesis is, however, opposed by several facts more closely discussed at page 166.

(2) The element might be attached to the plasm itself and with this might be transmitted to the progeny. Such a manner of inheritance through the plasm is formerly known from *Capsicum annuum* var. *albomaculata* (IKENO 1917) and from *Humulus Japonicus* var. *albomaculata* (WINGE 1919). In these cases it is a question of a plasmatic inheritance of white-mottling, and in such a way that the character in the former is transmitted both through eggs and through pollen, while in the latter only the eggs are able to transmit the mottling, as the male nucleus presumably does not carry plasm. As our blotching, as demonstrated above, is transmitted both through eggs and through pollen, a comparison with the conditions in *Capsicum annuum* would naturally suggest itself. There is, however, an essential difference between the way of inheritance of white-mottling in this plant and that of our blotching in oats, namely in the fact that all the progenies of a mottled *Capsicum* plant, — and it is a matter of indifference whether the plant is used as a father or as a mother —, become mottled and transmit the mottling to all the progenies in following generations, while in our case segregations (although not in Mendelian numerical relations) of both blotched and non-blotched progenies take place. This makes it unacceptable that the hereditary element of the blotching should be associated with the fluid plasm.

(3) So it seems that only one possibility is left, namely that the blotching-element is attached to the plastids of the plant.

If, then, a comparison with known cases of plastid inheritance both through eggs and through pollen is sought, only a single typical case presents itself, namely the inheritance of white-mottling in *Pelar-*

gonium zonale (BAUR 1908, 1911; for more recent literature see CHITTENDEN 1927). If here flowers of a green plant (or on green parts of a mottled plant) are pollinated with pollen from white shoots, — or reciprocally —, we always get mottled F_1 plants (these may, however, through a special distribution of the plastids be apparently quite white or quite green, which is in principle a subordinate question), and at the self-pollination of these, both green and mottled and white plants in non-Mendelian relations are segregated.

There is, of course, a difference in that our F_1 plants do not show any mosaic-like distribution of blotching-character.

We are, however, unable to give any safe explanation of the whole phenomenon; but it is perhaps permissible to conjecture that the plastids that carry the disposition to blotching, somehow or other badly influence the physiology of the whole plant, mainly in the youth of the plant, and that this disastrous influence just produces a local necrotisation of the tissue.

Our interpretation, that the blotching-character is transmitted by plastids, is further corroborated by the fact that the disease evidently is more easily inherited through eggs than through pollen, according to the fact that the male nucleus carries a far less quantity of cytoplasm (incl. quantity of plastids) than the female one.

To our knowledge there exists in the literature only a single case to be compared with our blotch leaf in oats, but this is evidently also perfectly in accordance with that of ours.

In 1923 R. A. EMERSON published a work entitled »The Inheritance of Blotch Leaf in Maize». The disease treated here presents the very same symptoms as our blotching in oats, and the conditions of inheritance are in principle the same, although EMERSON tries to bring them under the Mendelian scheme.

The last passages of EMERSON's summary run as follows:

»Blotch leaf is inherited as a recessive, or nearly recessive, character. If the blotched plants of the lowest grade, such as often occur in F_1, are grouped with the normals, F_2 progenies consist of normal and blotched plants in approximately a 3 : 1 ratio and backcross progenies in approximately a 1 : 1 ratio. These facts lead to the interpretation that blotch is due to the effect of a single pair of factors, *Bl bl*. The F_3 behavior is in accord with this hypothesis.

Great variations in the grade of blotch have been observed. Selection experiments with plants of diverse grades of blotch of F_2 and later generations show that these variations are inherited, at least in part. These results are interpreted as due to the action of modifying factors which are assumed to influence the intensity of blotch but which are without effect in the presence of the normal allelomorph of the blotch gene.»

We are unable to recognize the correctness of the conclusions which the author draws from his experimental results.

At the crossing of a blotched and a non-blotched variety of maize were in F_1 produced 711 non-blotched and 75 slightly blotched plants, and in F_2 was counted in 32 progenies of F_1 plants a total of 1043 non-blotched and 678 blotched plants. EMERSON draws from the outward appearance of F_1 the conclusion that non-blotched does not completely dominate over blotched, and tries to explain the F_2 segregation, 1043 : 678 as a 3 : 1 segregation, which, however, is disguised by the fact that a number of heterozygously blotched plants are not quite free from spots on the leaves, for which reason a too small number are counted among the dominating non-blotched group. He divides the grade of blotching into 7 groups and then gets the following distribution of the F_2 plants:

Blotch grade	0	1	2	3	4	5	6	7	Total
Number of F_2 plants...	1043	182	196	126	92	30	36	16	1721

EMERSON states that if plants belonging to the least blotched group (Grade 1) are enumerated as non-blotched (Grade 0) the blotched are to the non-blotched nearly as 3 : 1, as he finds the numbers 1225:496. In a similar way the numbers for backcross enumerations are corrected; at the backcrossing of F_1 to sound race 1050 non-blotched : 1381 blotched were produced, but in transferring the least blotched plants pertaining to grade 1, to the group of non-blotched he finds 1273 non-blotched and 1158 blotched plants, which is taken as an exponent of a 1 : 1 segregation.

As will be seen, the correction undertaken is very considerable, and it is of a decisive consequence whether it is permissible or not. As, however, a number of blotched plants are produced in F_1, not only of grade 1, but also of grade 2, it will be left to a subjective judgment how many of the slightly blotched plants of the F_2 generation should

be permitted to be transferred to the dominating, non-blotched group, and in this connection it is worth noting that considerably more than half of the blotched plants of the F_2 generation belongs to the slightly blotched, doubtful grades 1 and 2. While in F_1 only about 9,5 per cent were blotched, are now after the correction of the F_2 generation about 15 per cent of the plants, which were put down as non-blotched, really blotched, and in spite of this there is a deficit in the dominating group. as 1225 : 496 is not as 3 : 1, but as 2,47 : 1.

EMERSON's F_3 generation proves for the rest decisively that only part of the plants that show blotch grade 1 are heterozygously blotched; for 4 of a total of 10 proved homozygously blotched. Thus it cannot be permissible to transfer all plants of blotch grade 1 to the non-blotched.

Unfortunately it does not appear from EMERSON's work whether blotching is transmitted with equal easiness through both kinds of sex cells, as it is only stated that in some cases the disease is transmitted through pollen, in others through eggs.

If EMERSON's enumerations are treated without introduction of any correction, the correspondence with the inheritance of blotch leaf in oats is remarkable. Also EMERSON finds rather heterogeneous segregations in the F_3 and the F_4 generations, and by selection of greatly blotched plants constantly blotched families are gradually produced.

SUMMARY.

A characteristic spot necrosis, which occurs with a frequency of 100 per cent in a variety of oats originating from Nordfjord in Norway, has through crossing experiments with a sound oat (»Segerhavre») proved to be of a genetic nature. The disease, which is not due to any parasite nor in its nature is in accordance with virus diseases, is by us named »*Blotch Leaf*».

Blotch leaf is most easily transmitted through eggs, but also through pollen, to the progeny and further to the following generations. By selection in greatly blotched F_2 progenies and continued selection in F_3 it is possible to produce new, constantly blotched lines.

The segregations in F_2 and following generations are greatly varying and discordant to Mendelian numerical relations, for which reason the hereditary element in question cannot be associated with the chromosomes.

The hereditary element, which produces blotch leaf, is with the

greatest probability to be regarded as associated with the plastids of the variety.

Blotch leaf in oats is in its symptoms and in its way of inheritance almost completely in accordance with the blotch leaf in maize (investigated in 1923 by EMERSON), for which reason we must assume that EMERSON's interpretation: that blotch leaf in maize in principle shows simple Mendelian segregations, although disguised by incomplete relations of dominance, cannot be maintained.

EXPLANATION OF PLATES II AND III.

PLATE II.

Fig. 1. Leaf of the constantly blotched variety of oats originating from Nordfjord in Norway.

Fig. 2. Specimen of F_2 progeny from the F_1 hybrid: non-blotched variety (♀) × blotched variety (♂). Pedigree No. 2447.

PLATE III.

Fig. 3. Leaf of F_1 plant of the crossing blotched variety (♀) × non-blotched variety (♂).

Fig. 4. Specimen of F_2 progeny from the F_1 hybrid: blotched variety (♀) × non-blotched variety (♂). Pedigree No. 2453.

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